



Combined effects of elevated carbon dioxide and temperature on phytoplankton-zooplankton link: A multi-influence of climate change on freshwater planktonic communities

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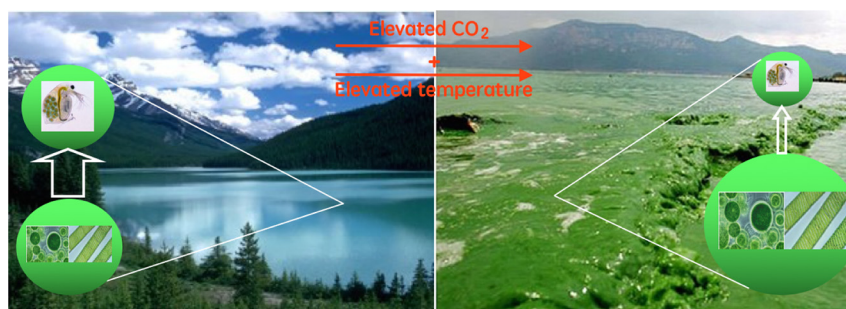
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HIGHLIGHTS

- Elevated CO₂ reduced phosphorus and ω 3 polyunsaturated fatty acids to carbon ratios.
- Elevated temperature exerted direct and indirect effect on zooplankton growth.
- Algae-mediated effect of elevated CO₂ indirectly reduced the growth of zooplankton.
- The growth of daphnids was mediated by ω 3 PUFAs/C ratio of algae.
- Elevated temperature can ameliorate the negative effects of elevated CO₂.

GRAPHICAL ABSTRACT



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ABSTRACT

It is essential to understand the combined effects of elevated CO₂ and temperature on phytoplankton-zooplankton link when attempting to predict climate change responses of freshwater ecosystems. Phytoplankton species differ in stoichiometric and fatty acids composition, and this may result in phytoplankton-mediated effect on zooplankton at elevated CO₂ and temperature. Beyond the isolated analysis of CO₂ or temperature effect, few studies have assessed zooplankton growth under the phytoplankton-mediated effects of elevated CO₂ and temperature. In this study, three algal species (green alga, diatom, cyanobacteria) were fed on zooplankton *Daphnia magna*, under the conditions of CO₂ concentrations of ambient (390 ppm) and elevated (1000 ppm) levels and temperatures at 20, 25 and 30 °C. Elevated CO₂ increased the algal biomass, while it reduced the phosphorus (P) and ω 3 polyunsaturated fatty acids (ω 3 PUFAs) to carbon (C) ratios. Elevated temperature decreased the P/C ratios in all algal cultures and ω 3 PUFAs/C ratios in the diatom and the cyanobacteria cultures. Phytoplankton-mediated effect of elevated CO₂ reduced the growth of zooplankton fed on the green and the mixed three algae culture. The stimulation of zooplankton fed on the diatom and the cyanobacteria by elevated temperature can be offset by decreasing food P and ω 3 PUFAs contents. The combined effects of elevated CO₂ and temperature on the growth of daphnids were mainly mediated by ω 3 PUFAs/C ratios in the phytoplankton. Rising temperature as a combined direct and indirectly phytoplankton-mediated effect on zooplankton may be able to ameliorate the negative effects of elevated CO₂. The results indicated that the combined effects of increased CO₂ and temperature increased the fatty acid content of the green alga but not the other algae. This study highlighted that climate change with simultaneously increasing temperature and CO₂ may entangle the carbon transfer in freshwater planktonic communities.

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1. Introduction

Climate change is arguably threatening to undermine freshwater ecosystems which have already been heavily exploited by humans. Climate change represents a complex compound of stressors, primarily an increase in temperature and elevated atmospheric CO₂ (IPCC, 2013). These two stressors can impact on freshwater ecosystems and those impacts that occur at lower trophic levels (e.g. primary producer) can cascade effect on other species through the food chain, which in turn affects ecosystem structure and function (Webb et al., 2008; Woodward et al., 2010). Emerging research illustrated that trophic interactions are especially strong candidates for biotic multipliers of climatic change (Zarnetske et al., 2012). For aquatic ecosystems, the phytoplankton-zooplankton interaction determines the structure of food web, which represents the energy and materials flowing through the population in the system (McQueen et al., 1989; Brett and Goldman, 1996, 1997). Hence, focusing on phytoplankton and zooplankton and their interactions is one path through the complexity of aquatic ecosystems.

Zooplankton herbivores will response to elevated CO₂ through phytoplankton-mediated effects (Allen et al., 2005; Urabe et al., 2003). Elevated CO₂ can increase photosynthetic efficiency and thus promote phytoplankton productivity (Schippers et al., 2004). Concomitantly, nutrient elements are diluted (Fu et al., 2007). Phosphorus, mostly contained in nucleic acids, is a limiting element for zooplankton herbivores. Decreases in phytoplankton phosphorus concentration and subsequently phosphorus to carbon (P/C) ratio under elevated CO₂ can therefore negatively impact zooplankton performance (Urabe et al., 2003; Urabe and Waki, 2009), potentially altering the carbon transfer between phytoplankton and zooplankton (Sterner, 1993; Sterner et al., 1993). In addition, fatty acids particularly polyunsaturated fatty acids, which are of critical to maintain zooplankton growth and reproduction, have been highlighted as the limitation factor for phytoplankton-zooplankton interactions (Müller-Navarra et al., 2000, 2003). Phytoplankton adjust their metabolisms to adapt to the high CO₂ condition, which results in the reallocation of cellular energy-rich molecules including PUFAs (Pronina et al., 1998; Muradyan et al., 2004; Hu and Gao, 2006). This implies that elevated CO₂ may also change the composition and decline the content of PUFAs in phytoplankton, slow zooplankton herbivore growth and thereby impede the carbon transfer between phytoplankton and zooplankton. However, this constraint exerted by phytoplankton PUFAs for zooplankton under elevated CO₂ has not been tested yet.

Elevated temperature has both direct and indirect impacts on phytoplankton-zooplankton interactions. Elevated temperature can lead to increasing photosynthetic rate and productivity of phytoplankton (Raven and Geider, 1988; Butterwick et al., 2005). It may exacerbate P dilution (Makino et al., 2011; Domis et al., 2014) and fatty acid saturation of phytoplankton (Sushchik et al., 2003; Jiang and Gao, 2004), reducing food quality and thus resulting in an indirect negative effect on zooplankton growth. Temperature can also have direct effects on zooplankton, such as stimulated consumption and growth (Lei and Armitage, 1980), which have a positive effect on zooplankton within its optimal temperature range (Orcutt and Porter, 1984). The trade off between the direct and indirect effects of temperature on zooplankton may be dependent on the constraints of P stoichiometry and/or fatty acids in phytoplankton. For example, PUFAs constraint on somatic growth of zooplankton deceased with increasing temperature, which suggested elevated temperature can mitigate the adverse effect of low PUFAs (Masclaux et al., 2009). Contrarily, increased temperature accelerated *Daphnia* growth while the effect of temperature was decreased by reduced P/C ratio, implying that direct stimulation of zooplankton growth by rising temperature can be offset by poor P content (Makino et al., 2011).

Elevated temperature and CO₂, as the most notable climate change, have occurred simultaneously rather than independently of each other. Previous studies only focus on a single climatic variable

(temperature or CO₂ concentration), which neglected the interactive effect between them (O'Neil et al., 2012). To evaluate the effect of climate change on freshwater ecosystems, it is necessary to consider how the interaction of elevated temperature and CO₂ impacts on the key trophic cascading of phytoplankton-zooplankton. Based on the above analyses, elevated temperature and CO₂ may decline P and/or PUFAs contents in phytoplankton, thereby indirectly aggravating to the growth of zooplankton. In contrary, elevated temperature can also directly stimulate the growth of zooplankton, which may ameliorate the indirectly negative effects of elevated temperature and CO₂. Thus, considering both temperature and CO₂ at simultaneously can enhance the predictions of the growth of zooplankton and the carbon transfer of phytoplankton-zooplankton coupling in the near future. Despite these, very few studies have investigated the combined effects of elevated temperature and CO₂ on the carbon transfer between phytoplankton and zooplankton. Predicting responses of phytoplankton-zooplankton coupling to climate change requires multi-factorial studies which better mimic the climatic conditions in the near future and understand the potential impact of climate change on freshwater ecosystems. The aim of this study was to quantify the combined effects of elevated temperature and CO₂ on the quality and the quantity of selected phytoplankton species, further evaluating this phytoplankton-mediated effect on the growth of zooplankton.

2. Materials and methods

2.1. Maintenance of algae and daphnids

Lake Kasumigaura is a typical shallow lake with the mean depth of 4 m. The second largest lake (171 km²) in Japan has been eutrophic since 1970's. Even after external inputs of nutrients were decreased since 1980's, water quality has not been improved due to accumulation of algae in water column. Climate change from elevated CO₂ and temperature has been experienced in the lake (Japan Meteorological Agency, 2008, 2012). Air CO₂ concentration around the lake have increased from 372.73 ppm in 1986 to 394.89 ppm in 1999, at the rate of 0.155 ppm per month ($R^2 = 0.54$, $p < 0.001$; Fig. 1S). Simultaneously, air temperature around the lake have increased since 1972, at the rate of 0.038 °C per year ($R^2 = 0.41$, $p < 0.001$; Fig. 2S). The explicit changes in CO₂ and temperature may influence the phytoplankton-zooplankton link, altering planktonic communities in the lake. Hence, the algal and daphnids species of the lake were isolated to study the response of the phytoplankton-zooplankton link to elevated CO₂ and temperature.

Three algal species which commonly co-exist in the lake, *Scenedesmus acuminatus* (green alga), *Cyclotella meneghiniana* (diatom) and *Microcystis aeruginosa* (cyanobacteria), were isolated from the lake water with total phosphorus concentration of 0.1 mg·L⁻¹ and total nitrogen concentration of 1.1 mg·L⁻¹. These taxa represent major groups of freshwater phytoplankton and widely distributed in lakes all over the world (Brönmark and Hansson, 2005). These species can also be classified to three major functional groups of lake algae. The same functional group has the similar biochemical compositions (e.g. P stoichiometry, fatty acids), reflecting the approximate bottom-up effect on higher trophic levels (e.g. zooplankton, fish) (Urabe and Watanabe, 1992; Müller-Navarra et al., 2000; Reynolds et al., 2002). These algae species with the same cell dimension of 2–4 µm were chosen to feed *Daphnia* in this work, which can exclude the effect of food size on the prey of zooplankton. After isolated, the axenic strains were stored by using a certain medium (CS, CSi and MA medium for green alga, diatom and cyanobacteria, respectively), subsequently culturing under 20 °C and 15 µmol photons m⁻² s⁻¹ flux density on a 10/14 h light/dark cycle. During this experiment, three algal species were cultivated in COMBO medium to exclude the effect of medium. The medium has similar components with lake water, supporting robust growth and reproduction of planktonic organisms (Kilham et al., 1998).

Daphnids species usually occurs in many lakes and dominates the zooplankton communities around the world (Wojtal-Frankiewicz, 2012). Together with its strong top-down control to algae, it has been widely used for a model organism to predict the potential response of planktonic communities to changing environments. Daphnids species are also widely used in ecotoxicology studies as a standard reference for other non-daphnids zooplankton (Miner et al., 2012). Daphnids species was usually investigated in Lake Kasumigaura. Thus, these algal and daphnids species can mimic the phytoplankton-zooplankton link in the lake. Zooplankton *Daphnia magna* was obtained from the National Institute for Environmental Studies Collection. This species was maintained in the culture collection for 5 years under constant conditions. All clones were cultured in a nutrient-free COMBO medium by 5 mgC L^{-1} *Scenedesmus acuminatus* suspension. The culture was maintained in a thermostatic room where temperature was set to 20 ± 0.1 °C. Before the experiment, to produce homogenous offspring from a single female, one female *Daphnia* with clutch of eggs was picked into another beaker for enrichment culture under the same conditions.

2.2. Experimental setup

Atmospheric concentration of CO_2 is expected to continue to rise to 540–970 ppm from the present until the year of 2100, at which global air temperature will be increased by 2–5 °C (IPCC, 2013). Accordingly, in this study, temperature was treated at 20, 25 and 30 °C under two CO_2 partial pressures (390 and 1000 ppm) to simulate climate change during this century (IPCC, 2013).

CO_2 and temperature exposure experiments were performed in a microcosm system in which algae and daphnids were cultured by continuous medium flow. Before experiment, all utensils of the microcosm components and COMBO medium were autoclaved to prevent bacterial infection. The microcosm system was mainly composed of two chemostates in which algae and daphnids were cultivated respectively. The data obtained from the first chemostat was used to measure the biomass, the P and $\omega 3$ PUFAs to carbon ratio of algae. The data from the second one was used to calculate the growth rate of *Daphnia*.

The sterile medium was introduced into a 2 L glass flask (the first chemostat) at the rate of $150 \text{ mL} \cdot \text{d}^{-1}$. Standard air with appropriate partial pressure of CO_2 from gas cylinder was also injected into the flask through an air inlet tube connected with a $0.2 \mu\text{m}$ pore size filter. The output of medium with algal cells was siphoned from the flask by a glass tube installed on the surface of medium. The rate of medium output equaled that of input, which maintained the medium volume of 1.5 L. The pure culture of the three algal species and their mixed culture inoculated together were performed over a period of 17 days and conducted in triplicate. The initial algal density for each culture was $3.5 \times 10^4 \text{ cells L}^{-1}$.

When algal density in the first chemostat reached a saturation level after 9 days, algal suspension was siphoned into another chamber (the second chemostat) at a rate of $150 \text{ mL} \cdot \text{d}^{-1}$ by a tube connected with two chambers. At 12 days, 30 neonates of *Daphnia magna* with 3 days of birth were introduced into the second chamber where 450 ml algal medium were contained. *Daphnia* were remained in the chamber for 5 days. During the 5-day period, algal suspension in the chamber continued to be drawn off at a rate of $150 \text{ mL} \cdot \text{d}^{-1}$ but daphnids were retained, which maintained the medium volume of 450 ml. At the end of experiments (17 days), daphnids individuals in the chemostat were collected.

Both chambers with and without daphnids were connected with each other and set in water bath that kept phytoplankton and zooplankton exposed to the same CO_2 and temperature. Fluorescent lamps were set on the surface of water bath to supply light at photon fluence rates of $60 \mu\text{mol E m}^{-2} \text{ s}^{-1}$ with a 12/12 h light/dark cycle.

2.3. Analysis for algal biomass and daphnids growth

At 12, 15 and 17 days, a 50 ml aliquot containing algae was sampled from the chamber without daphnids and freeze dried. Then, the total organic carbon of algae was measured with carbon analyzer (TOC-SSM5000, Shimadzu, Kyoto, Japan). The mean total organic carbon of algae at 12, 15 and 17 days was regarded as the input of algal biomass into the chamber with daphnids.

Subsamples of *Daphnia* neonates were employed to estimate initial body mass before being introduced into the chamber. *Daphnia* individuals in the second chemostat at the end of experiment were collected to measure their dry mass. Growth rate of individuals was estimated by assuming exponential increase in body mass (Elser et al., 2001). The growth rate was determined as

$$g = \frac{\ln W_t - \ln W_0}{t}$$

where W is the mean body mass of *Daphnia* at the beginning (W_0) and end (W_t) of the experimental duration of $t = 5$.

2.4. Analysis for phosphorus and fatty acids of algae

Concentrations of algal P were measured by using the algal suspensions collected from the first chamber without daphnids. The suspension was filtered onto glass fiber filter with $0.45 \mu\text{m}$ pore size and analyzed by using spectrophotometry after persulphate oxidation (APHA, 1992).

Fatty acids contents of algae and daphnids were analyzed by one-step method (Abdulkadir and Tsuchiya, 2008). Sample freeze dried for 24 h were mixed with 4 ml of hexane and 1 ml of internal standard solution (1 mg tricosanic acid was dissolved in 100 ml hexane) and added with 2 ml of 14% BF_3 in methanol. After heated at 100 °C for 120 min, sample was successively added with 1 ml of hexane and 2 ml of distilled water. Then, sample was centrifuged to extract two liquid phases. The upper hexane layer was injected into GC mechanism (GC-2014, Shimadzu, Kyoto, Japan) for fatty acid methyl esters analysis. Fatty acid concentrations were estimated by comparing the peak area of fatty acid in sample with that of internal standard as follows:

$$C_{FA} = \frac{A_S}{A_{IS}} \times \frac{C_{IS}}{W_S}$$

where A_S is the chromatographic peak area of a certain fatty acid in sample; A_{IS} is the chromatographic peak area of the internal standard; C_{IS} is the concentration of the internal standard (mg); W_S is the weight of sample (g). PUFAs, particularly eicosapentaenoic acid (EPA), docosahexenoic acid (DHA), linolenic acid (SDA) and eicosatetraenoic acid (ARA), are critical to carbon transfer between primary producers and consumers, as they are necessary compounds for the growth of consumers (Müller-Navarra et al., 2003). Thus, in this study, the analysis of fatty acids focused on these PUFAs species.

2.5. Data analysis

The variance analysis of two-way ANOVA was conducted to investigate the following effects: temperature, CO_2 level and the interactive effect between temperature and CO_2 . The post hoc Tukey's multiple comparison test was used to discriminate the significant differences among elevated temperature and CO_2 treatments. The regression analyses between *Daphnia* growth rate and P or $\omega 3$ PUFAs to carbon ratio were fitted by logistic model (Müller-Navarra et al., 2000). The generalized linear model (GLM) with selection procedures was employed to evaluate the relative importance of factors related to *Daphnia* growth. In this analysis, the growth rate of *Daphnia* was set as the response variable and temperature, CO_2 , algal biomass, P and $\omega 3$ PUFAs to carbon ratio were set as the explanatory variables. For small data sets as this

study, a second-order variant of the Akaike information criterion (AICc) was employed to determine the best model. AICc is a valid criterion for a linear model with fixed effects under Gamma assumption. The Akaike's weights were used to define the relative importance of each explanatory variable across the full set of models evaluated by summing $\Delta AICc$ values of all models that include the variable of interest, taking into account the number of models in which each variable appears (Burnham and Anderson, 2002). All analyses were conducted by using SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA) with untransformed data.

3. Results

3.1. Phytoplankton responses to elevated CO₂ and temperature

Both CO₂ and temperature significantly impacted the biomass of the green alga, the cyanobacteria and the mixed culture (Table 1). At a given temperature, the biomass at high CO₂ cultivation was higher than that at ambient CO₂, which indicated elevated CO₂ promoted the production of these algae. The biomass of these algae increased with temperature at high CO₂ level (Fig. 1). At elevated CO₂, the biomass of the green alga increased from 9.36 to 16.4 mgC L⁻¹ with increasing temperature from 20 to 30 °C, correspondingly from 3.88 to 13.02 mgC L⁻¹ for the cyanobacteria. The impact of temperature on the biomass of the mixed culture was similar to the green alga, due to the dominance of the green alga in all treatments (Li et al., 2016). With rising temperature, the biomass of the mixed culture decreased from 8.65 to 5.95 mgC L⁻¹ at ambient CO₂, while increased from 9.91 to 12.76 mgC L⁻¹ at elevated CO₂. The diatom reached to its maximal biomass at 25 °C, while elevated CO₂ had no significant effect on its growth ($p = 0.063$). Significant interaction between CO₂ and temperature was observed for the biomass of overall algae cultures (the factor of CO₂ × Temperature, Table 1).

Both CO₂ and temperature significantly impacted the P/C ratios of overall algae cultures (Table 1). Elevated CO₂ decreased the P/C ratios at a certain temperature. At both CO₂ levels, elevated temperature decreased the P/C ratios of algae cultures except the diatom (Fig. 2). For the diatom, the P/C ratio decreased from 0.896×10^{-2} to 0.765×10^{-2} at 20 °C, from 0.906×10^{-2} to 0.859×10^{-2} at 25 °C, and 0.879×10^{-2} to 0.800×10^{-2} at 30 °C as CO₂ increased from 390 to 1000 ppm. The effect of the interaction between CO₂ and temperature on the P/C ratio of the green alga and the mixed culture was detected (Table 1), which indicated elevated CO₂ and temperature can aggravate the decline of the P/C ratio.

Both CO₂ and temperature with their interaction effect significantly impacted the $\omega 3$ PUFAs/C ratios of overall algae cultures (Table 1). The $\omega 3$ PUFAs/C ratios of the green alga and the mixed culture increased with rising temperature, while decreased with CO₂. The extent of elimination at 30 °C was greater than that at 20 °C. At 390 ppm CO₂, the $\omega 3$ PUFAs/C ratio of the green alga increased from 0.136 to 0.325 mg·mg⁻¹ by increasing temperature from 20 to 30 °C, respectively from 0.102 to 0.215 mg·mg⁻¹ at 1000 ppm CO₂. The $\omega 3$ PUFAs/C ratio of the mixed culture increased from 0.117 to 0.270 mg·mg⁻¹ with temperature increasing from 20 to 30 °C, respectively from 0.102 to 0.205 mg·mg⁻¹ at 1000 ppm CO₂. The $\omega 3$ PUFAs/C ratio of the diatom decreased with rising temperature for both CO₂ levels. The $\omega 3$ PUFAs/C ratio of the diatom decreased from 0.139 to 0.125 at 20 °C, from 0.109 to 0.078 at 25 °C, and from 0.070 to 0.066 at 30 °C as CO₂ increased from 390 to 1000 ppm. The lowest $\omega 3$ PUFAs/C ratio of the cyanobacteria was observed at 30 °C and high CO₂ level (Fig. 3). The effect of the interaction between CO₂ and temperature on the $\omega 3$ PUFAs/C ratio of all algal cultures were detected (Table 1), which indicated elevated CO₂ and temperature can aggravate the decline of the $\omega 3$ PUFAs/C ratio. Additionally, EPS and DHA were not detected in the green alga and the cyanobacteria in all temperature and CO₂ elevation treatments (data not shown).

Table 1
Two-way ANOVA results of analysis of variance for P/C ratio and $\omega 3$ PUFAs content of algae, and growth rate of daphnid fed on algae treated by elevated temperature and CO₂. Values given are F and p statistics for $n = 3$ replicates.

Factors	d. f.	Biomass							
		Green alga		Diatom		Cyanobacteria		Mixed culture	
		$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p
CO ₂	1	237.036	<0.001*	4.2	0.063	91.135	<0.001*	200.484	<0.001*
Temperature	2	19.356	<0.001*	43.660	<0.001*	160.549	<0.001*	3.950	0.048*
CO ₂ × temperature	2	47.487	<0.001*	5.380	0.021*	33.097	<0.001*	34.754	<0.001*
Factors	d. f.	P/C ratio							
		Green alga		Diatom		Cyanobacteria		Mixed culture	
		$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p
CO ₂	1	118.798	<0.001*	33.082	<0.001*	35.957	<0.001*	115.338	<0.001*
Temperature	2	61.590	<0.001*	4.669	0.032*	103.817	<0.001*	32.598	<0.001*
CO ₂ × temperature	2	10.227	0.003*	2.766	0.103	0.257	0.778	4.442	0.036*
Factors	d. f.	$\omega 3$ PUFAs/C ratio							
		Green alga		Diatom		Cyanobacteria		Mixed culture	
		$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p
CO ₂	1	103.852	<0.001*	19.030	0.001*	21.899	0.001*	13.326	0.003*
Temperature	2	193.838	<0.001*	96.752	<0.001*	19.759	<0.001*	124.478	<0.001*
CO ₂ × temperature	2	13.516	0.001*	4.287	0.039*	7.389	0.008*	6.466	0.012*
Factors	d. f.	Growth rate of <i>Daphnia</i>							
		Green alga		Diatom		Cyanobacteria		Mixed culture	
		$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p	$F_{5,17}$	p
CO ₂	1	12.845	0.004*	4.294	0.060	1.235	0.288	5.342	0.039*
Temperature	2	16.800	<0.001*	25.512	<0.001*	7.429	0.008*	7.548	0.008*
CO ₂ × temperature	2	0.110	0.897	2.559	0.119	5.640	0.835	4.042	0.046*

The symbol (*) denotes significant p value ($p < 0.05$).

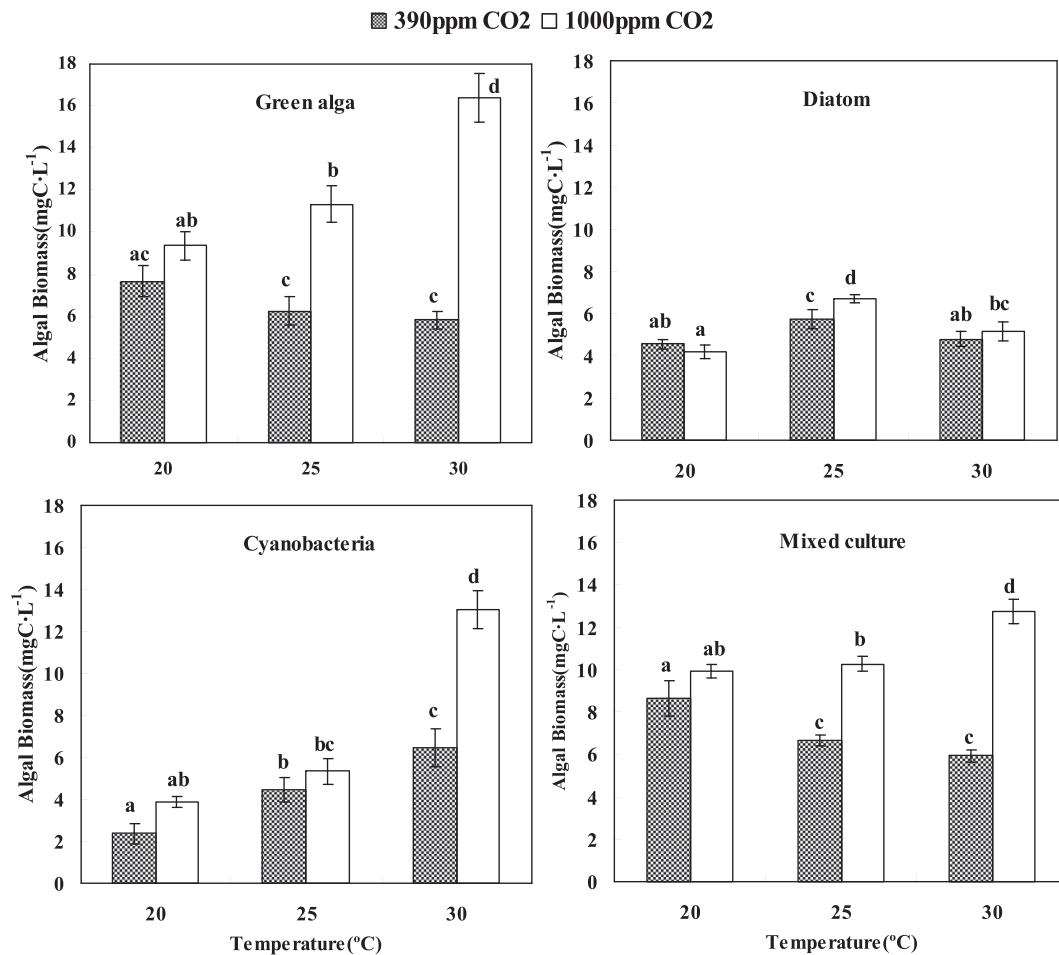


Fig. 1. Biomass of algae at steady state by elevated temperature and CO₂ treatments.

3.2. Zooplankton responses to elevated CO₂ and temperature

Both CO₂ and temperature significantly impacted the growth rate of *Daphnia* fed on the green alga (Table 1). The growth rate of *Daphnia* fed on the green alga increased with elevated temperature for both CO₂ levels, while decreased with elevated CO₂. At 390 ppm CO₂, the growth rate of *Daphnia* fed on the green alga was increased from 0.503 to 0.592 by increasing temperature from 20 to 30 °C, and from 0.468 to 0.543 d⁻¹ at 1000 ppm CO₂. No significant interaction effect between CO₂ and temperature was observed on the growth of *Daphnia* fed on the green alga. Only temperature significantly impacted the growth rate of *Daphnia* fed on the diatom and the cyanobacteria (Table 1). The minimal growth rates of *Daphnia* fed on the diatom and the cyanobacteria were observed at 30 °C. The minimal growth rates of *Daphnia* fed on the diatom were 0.455 d⁻¹ at 390 ppm CO₂ and 0.415 d⁻¹ at 1000 ppm CO₂, correspondingly 0.076 and 0.038 d⁻¹ for *Daphnia* fed on the cyanobacteria. Both CO₂ and temperature with their interaction effect significantly impacted the growth rate of *Daphnia* fed on the mixed culture (Table 1). The growth rate of *Daphnia* fed on the mixed culture was increased from 0.483 to 0.587 by elevated temperature at ambient CO₂ level. However, elevated temperature had no effect on the growth rate of *Daphnia* fed on the mixed algae at high CO₂ level, which indicated the effect of temperature was vanished by elevated CO₂.

The best GLM excluded algal biomass while selected temperature, CO₂, P/C and ω3 PUFAs/C ratio as explanatory variables (AICc = -100.165, Table 2), which indicated algal biomass have no significant effect on the growth of *Daphnia*. The best model selected temperature

as a positive factor while CO₂ as a negative factor, which indicated elevated temperature stimulate while elevated CO₂ inhibit the growth of *Daphnia*. Excluding the algal P/C and ω3 PUFAs/C ratios from the best model resulted in essentially unsupported model, judging from a large increase in AICc (ΔAICc = 9.343 for P/C ratio and 69.754 for ω3 PUFAs/C ratio, Table 2), indicating the indirect effects of elevated temperature and CO₂ on the growth of *Daphnia* were mediated by the qualitative traits of algae. Moreover, the Akaike weights for each variable across all the models in which it appeared were summed to estimate the relative importance of each variable for the growth of *Daphnia*; the larger this sum, the more important a given variable is relative to the other variables used in the same models. By this criterion, the two most important variables of were the algal ω3 PUFAs/C and P/C ratios, while the least contribution of was algal biomass (Fig. 5). Given assigning 1 unit to the most contribution of the ω3 PUFAs/C ratios, the importance of the P/C ratios was 0.88 unit while algal biomass only contributed to 0.26 unit. The growth rate of *Daphnia* was positively correlated with P/C ($y = 0.559[1 - \exp.(-400.528x + 2.155)]$), $p = 0.023$, $R^2 = 0.393$; Fig. 6.a) and ω3 PUFAs/C ratio ($y = 0.578[1 - \exp.(-21.363x + 0.3767)]$), $p = 0.003$, $R^2 = 0.914$; Fig. 6.b).

4. Discussion

Elevated temperature stimulated the growth of zooplankton fed on the good quality of algal food. In this study, the growth of *Daphnia* fed on the good quality (high ω3 PUFAs/C or P/C ratio) algal cultures (the green alga and the mixed culture) were increased by increasing

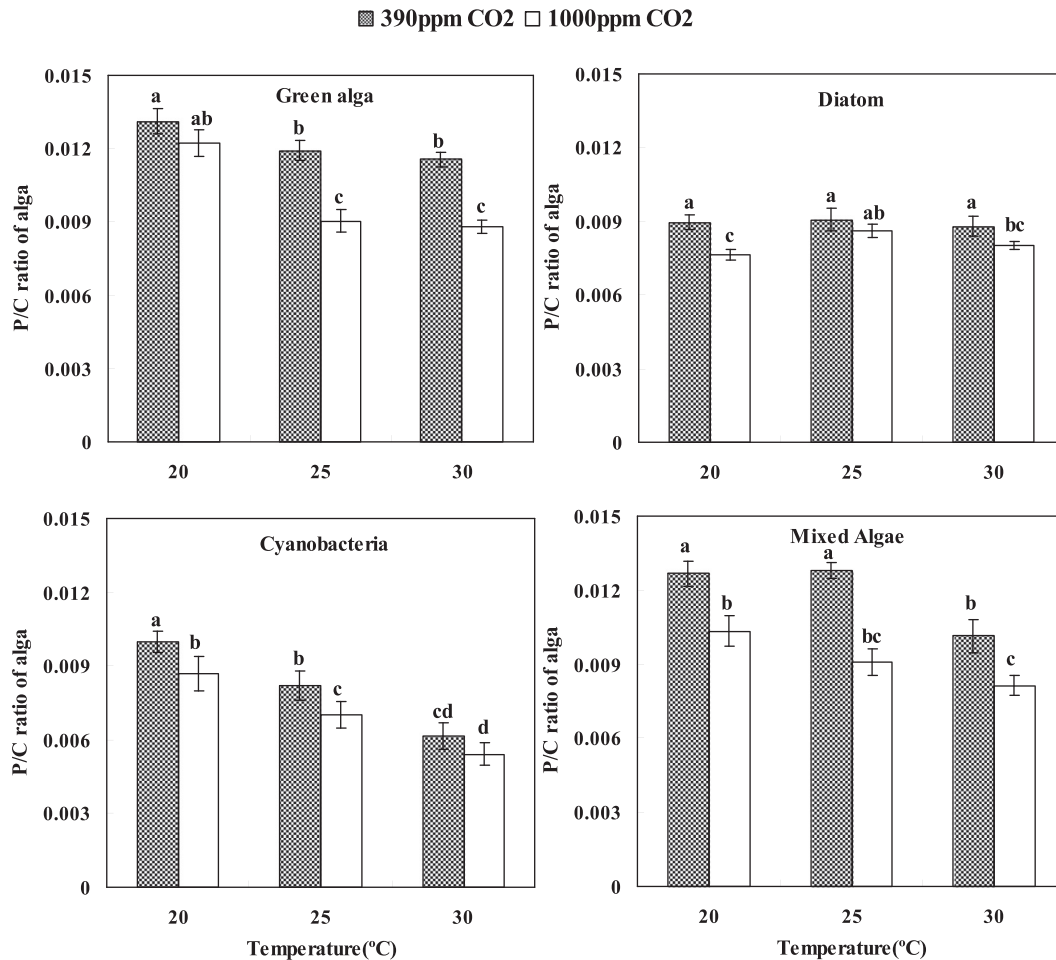


Fig. 2. P/C ratio of algae by elevated temperature and CO₂ treatments.

temperature from 20 to 30 °C, implying a positive effect of temperature on daphnids (Figs. 2–4). The growth rate of daphnids species increased with rising temperature across a wide range (e.g., 5–30 °C, Lei and Armitage, 1980, Orcutt and Porter, 1984), even though some species exhibited the maximal growth rate at around 20–30 °C (e.g., Mitchell and Lampert, 2000; Giebelhausen and Lampert, 2001). However, the positive effect of elevated temperature may be offset by the reduction of algal quality. Compared with the green algae and the mixed culture, ω 3 PUFAs/C and P/C ratio in the diatom and the cyanobacteria were lower, which may constrain the growth of zooplankton (Persson et al., 2011; Masclaux et al., 2009). Increasing temperature further reduced ω 3 PUFAs/C and P/C ratios in these two algal cultures, resulting in the decrease of the daphnids growth with elevated temperature (Figs. 2–4). It needs to highlight the fact that the effect of food quantity was not identical in the current study setup. Although no significant effect of algal biomass on the growth of *Daphnia* was detected in all treatments (Table 2), further study in the future will be required to directly investigate the effect of food quantity vs. quality.

Recent studies have also analyzed indirect versus direct effects of elevated temperature on the growth of *Daphnia*. When temperature increased from 16 to 30 °C, there was no direct effect of elevated temperature on daphnids growth, but indirect negative effect on daphnids growth via decreasing algal PUFA content (Sikora et al., 2014). In this study, the effect of temperature on the growth rate of *Daphnia* was also dependent on the food quality fed to *Daphnia*. The best GLM selected P/C and ω 3 PUFAs/C ratios accompanied with temperature as meaningful factors in the large variation of *Daphnia* growth rates among treatments (Table 2). The growth rate of *Daphnia* fed on the green algae or the mixed culture increased with temperature

continuously from 20 to 30 °C at 390 ppm CO₂, while the growth of *Daphnia* fed on the diatom or the cyanobacteria could not continue to increase with increasing temperature (Fig. 4). These results implied that temperature not only directly affected daphnids growth but also indirectly through altering algal food quality (P/C and/or ω 3 PUFAs/C ratios). Moreover, the direct effect of increasing temperature was observed that food quality constraints particularly poor EPA and SDA on somatic growths of two zooplankton species ameliorated by increasing temperature from 12 to 25 °C (Masclaux et al., 2009). In contrast to this finding, the direct stimulation of warming to zooplankton growth can be offset by decreasing algal food quality. When P/C and ω 3 PUFAs/C ratios of the diatom or the cyanobacteria were further decreased by increasing temperature to 30 °C, the growth of daphnids decreased (Fig. 4).

Zooplankton may experience indirect phytoplankton effect as a result of elevated CO₂. Previous study showed CO₂ does not directly affect *Daphnia* performance if it was within the range between 350 and 3500 ppm (Urabe et al., 2003). When algae were cultured at high CO₂ level (2000 ppm), algal abundance increased but algal P/C ratio decreased which indirectly resulted *Daphnia* decreased growth rates (Urabe and Waki, 2009). In this study, the growth of *Daphnia* fed on the green algae or the mixed culture decreased by a decrease in P/C and ω 3 PUFAs/C ratios at 1000 ppm CO₂ (Figs. 2 and 3). Together, CO₂ as a negative factor with P/C and ω 3 PUFAs/C ratio was selected to explain the daphnids growth in the best GLM. Consistent with previous studies, these results indicated that elevated CO₂ indirectly decreased zooplankton growth via reducing food quality. However, the effect of elevated CO₂ on *Daphnia* fed on the diatom and the cyanobacteria was not observed (Fig. 4, Table 1). The variations of P and ω 3 PUFAs with CO₂ in

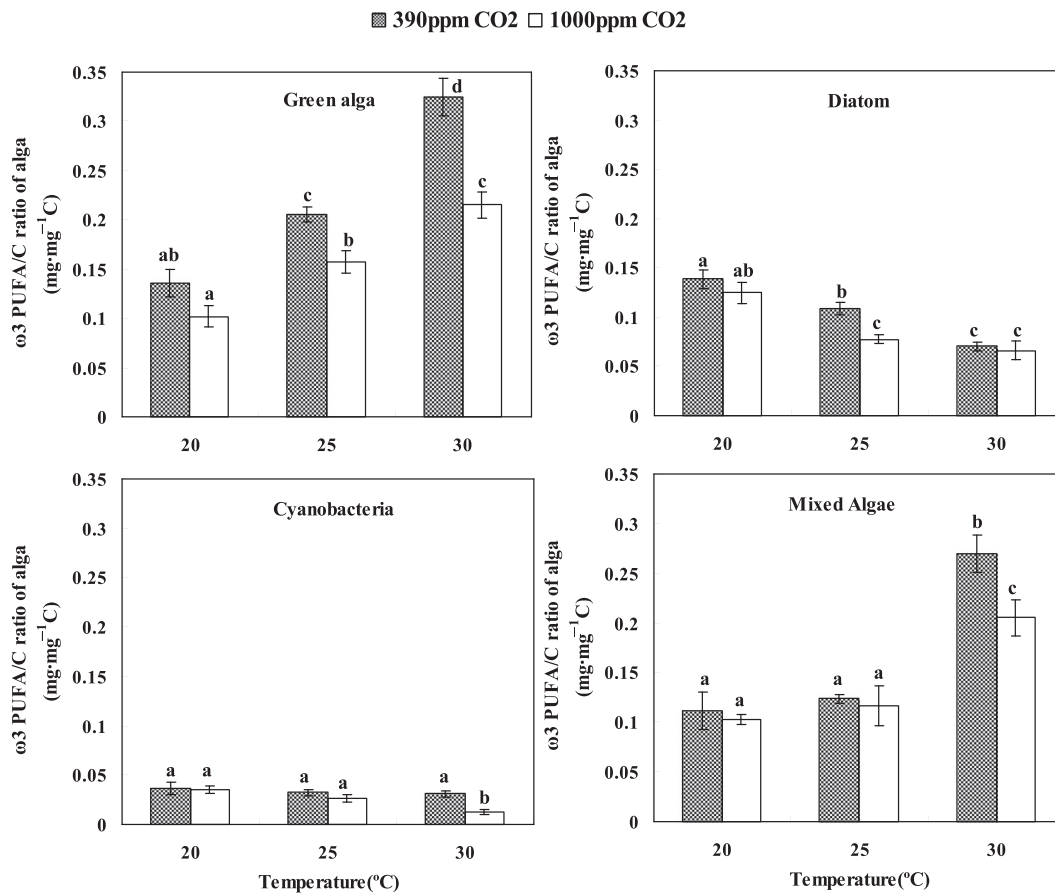


Fig. 3. $\omega 3$ PUFAs content of algae by elevated temperature and CO₂ treatments.

the diatom and the cyanobacteria were less than the green algae and the mixed culture, which may conceal the response of *Daphnia* fed on the diatom or the cyanobacteria at elevated CO₂. In addition, the high CO₂ (1000 ppm) used in this study coincided with the atmospheric levels predicted to be probably reached by 2100 year (Intergovernmental panel on climate change, 2013), while two times lower than previous studies (2000 ppm in Urabe et al., 2003; Urabe and Waki, 2009). The unrealistic high CO₂ level in previous studies may strikingly increase the variable in algal quality and the response of zooplankton.

Beyond the isolated analysis of temperature or CO₂ effect, few studies assessed the zooplankton's growth under the phytoplankton-mediated effects of elevated temperature and CO₂ and the direct effect of elevated temperature. In this study, an interactive effect of temperature and CO₂ was only observed with *Daphnia* fed on the mixed culture. Although the growth rate of *Daphnia* fed on the mixed culture increased as a result of elevated temperature, it compensated fully for the decreased growth attributed to the phytoplankton-mediated effect of elevated CO₂. Algal composition in the mixed culture was the result of

competition among algal species under the culture conditions that were, in this study, designed to rising temperature and CO₂ levels in eutrophic lake. Due to the different responses of algal species (in the monocultures) to temperature and CO₂, the biomass proportions of the green algae and the diatom in the mixed culture decreased with increasing temperature and CO₂ while the biomass of the cyanobacteria increased (Li et al., 2016). The changes in algal composition could also impact nutrient stoichiometry and fatty acids composition in the mixed culture, which further determines the combined effect of elevated temperature and CO₂ on zooplankton.

Even though temperature and CO₂ were elevated simultaneously, $\omega 3$ PUFAs/C ratio was determinant of phytoplankton food quality for herbivorous zooplankton (Figs. 5 and 6.b). The growth rate of *Daphnia* increased with increasing $\omega 3$ PUFAs/C ratio. It is generally believed that EPA and DHA, which maintain cellular membrane structure and function, are the most important long-chain (C20–C22) PUFA in vertebrates and invertebrates. The positive correlations of the growth of *Daphnia* to EPA or DHA concentration of phytoplankton species were found in the

Table 2

Results of model selection based on the generalized linear model for growth rate of daphnid.

AICc	Δ AICc	Residual deviance	Estimated coefficient for explanatory variable				
			Temperature	CO ₂	$\omega 3$ PUFAs/C ratio	P/C ratio	Biomass
−100.165	0	8.233	0.008	−0.049	3.331	32.168	
−97.714	2.451	8.232	0.009	−0.049	3.336	31.840	0.001
−90.822	9.343	9.658	−0.001	−0.019	4.114		
−90.147	10.018	9.436	0.001	−0.036	4.050		−0.004
−30.411	69.754	21.756	0.004	−0.091		101.264	
−29.143	71.022	21.436	0.002	−0.053		106.526	0.007
0.547	100.712	32.664	0.004	0.086			3.190
2.635	102.80	34.582	−0.001	0.029			

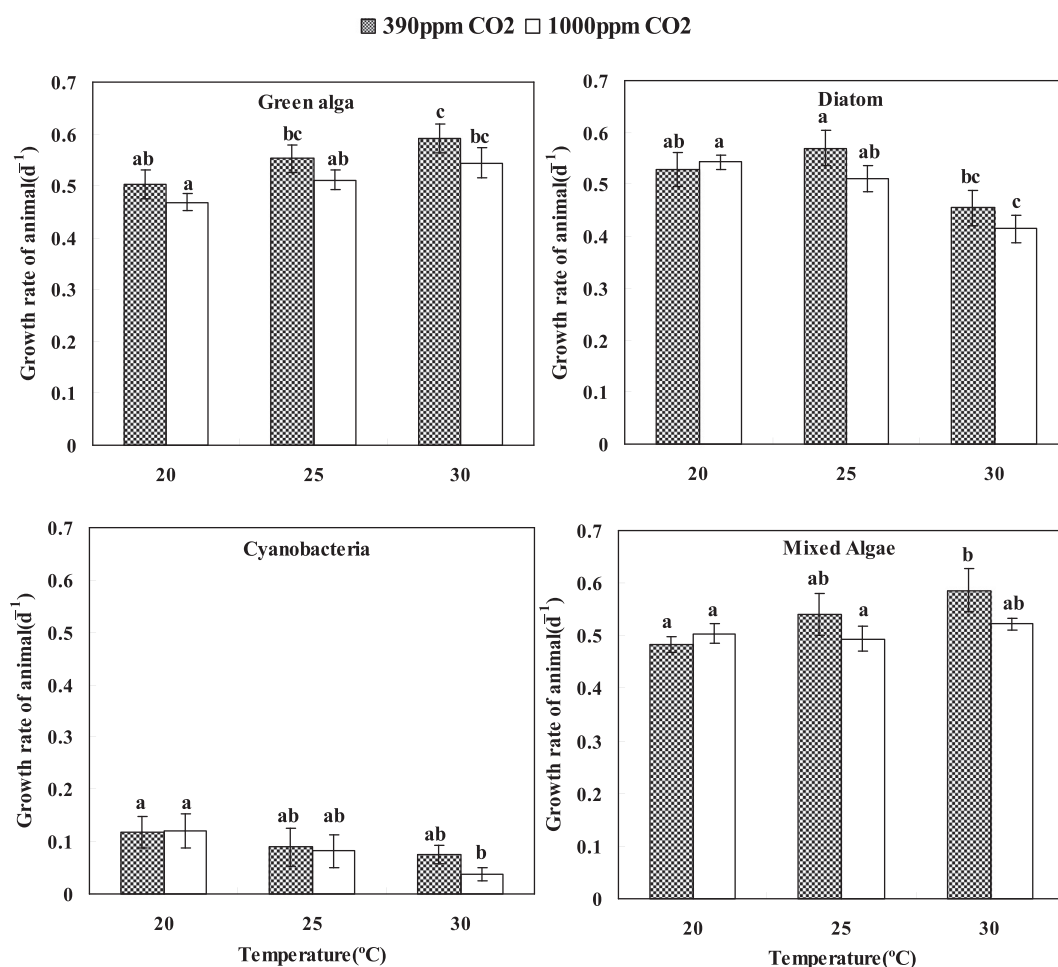


Fig. 4. Growth rate of daphnid fed on algae treated by elevated temperature and CO₂ treatments.

laboratory (Weers and Gulati, 1997) and field studies (Müller-Navarra et al., 2000, 2003). The quality of algae as food for *Daphnia* decreased due to the reduced amount of EPA and DHA. These compounds were mainly food derived and could not be synthesized de novo by most animals (Bychek et al., 2005). Contrary to the previous studies, EPA or DHA to carbon ratio was not related to *Daphnia* growth in this study. Neither the green alga nor the cyanobacteria contained EPS and DHA in all temperature and CO₂ elevation treatments. *Daphnia* grown well as fed on

the green alga (0.482–0.609 d⁻¹), whereas grown badly if fed on the cyanobacteria (0.025–0.145 d⁻¹). Green alga *Scenedesmus obliquus* without EPA and DHA was also reported as a good-quality food to deal with zooplankton development in previous studies (Giebelhausen and Lampert, 2001; Sikora et al., 2014). The growth disparity between daphnids fed on different free-EPA and DHA algae suggested that the content of EPA and DHA in phytoplankton was not a complete determinant for the growth of zooplankton. This result was confirmed by recent studies which found C18 PUFAs (in particular ARA and SDA) were elongated and desaturated to yield longer carbon chain EPA by daphnids (Taipale et al., 2011). When sufficient quantities of C18 ω3 PUFAs (ARA and SDA) precursors were available (i.e. 0.122–0.344 mg·mg⁻¹C of ARA and SDA in the green alga of this study), EPA biosynthesis of daphnids was sufficient for optimizing its growth. Therefore, ω3 PUFAs included ARA, SDA, EPA and DHA were the fundamental fatty acids for *Daphnia* growth.

The constraint of P on daphnids was weaker than that of ω3 PUFAs (Table 2; Figs. 5 and 6). The P/C ratio of algae was positively but weakly correlated to daphnid growth ($R^2 = 0.393$, $p = 0.023$), compared with ω3 PUFAs/C ratio ($R^2 = 0.914$, $p = 0.003$). P-based stoichiometric constraint on consumer growth was often observed in recent years. These experiments supplied a gradient of P/C algae to *Daphnia*, stimulated animal growth and demonstrated clearly that P deficiency could constrain the growth rate of consumers (Sterner and Hessen, 1994; Elser et al., 2001). The element stoichiometry depends on the availability of the element in ecosystems. In this study, phytoplankton species were not limited by P which was abundant in the medium. The minimal P/C molar ratios (the cyanobacteria at 30 °C and 1000 ppm CO₂) even reached

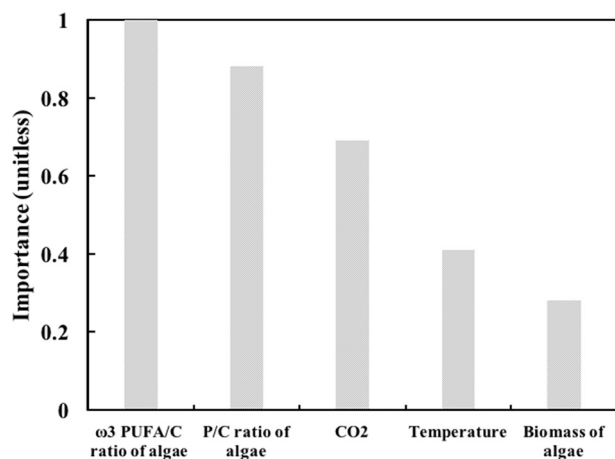


Fig. 5. Relative importance of variables in the generalized linear model for growth rate of daphnids.

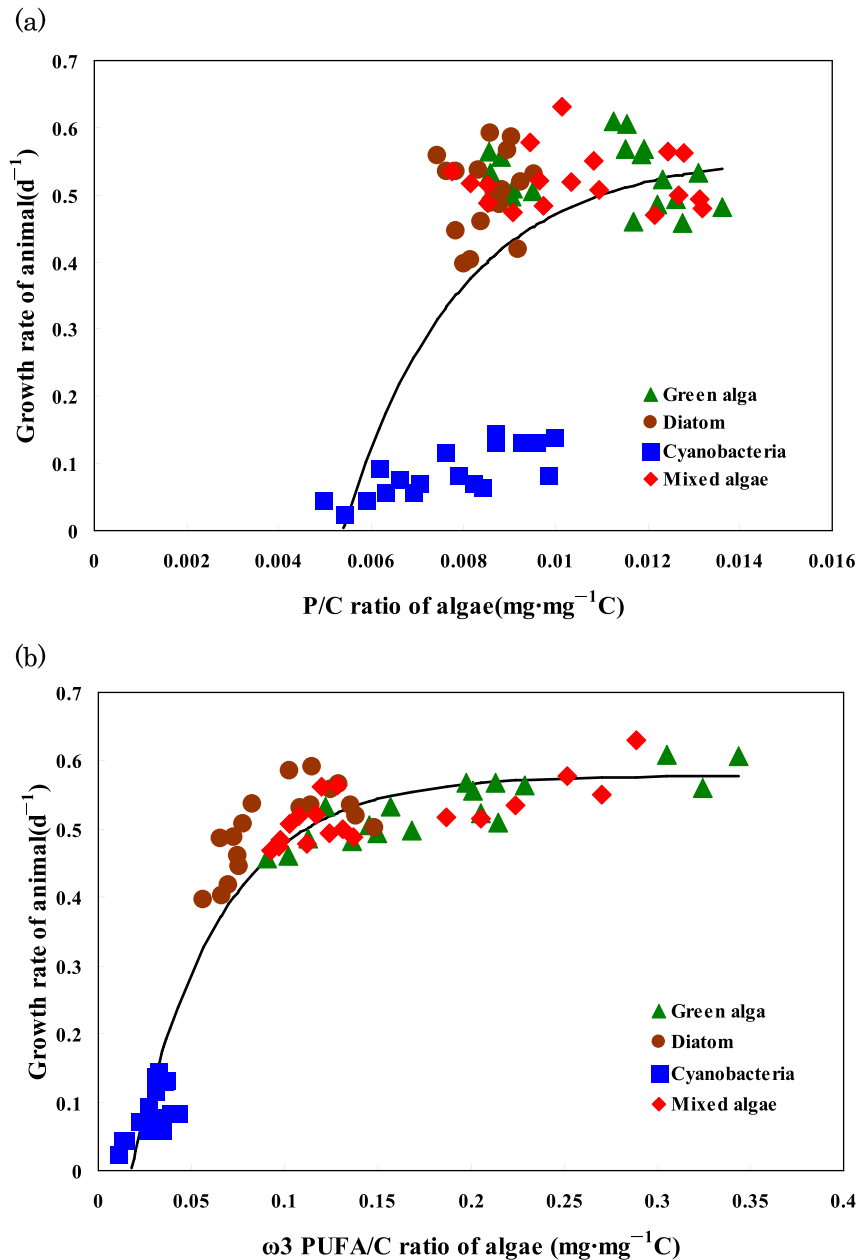


Fig. 6. Relationship between (a) the molar phosphorus to carbon ratio and daphnid growth rate; (b) the molar ω3 PUFAs to carbon ratio and daphnid growth rate.

$5.0 \times 10^{-3} \text{ mg} \cdot \text{mg}^{-1} \text{C}$ which was higher than the P limitation for *Daphnia* growth occurred at P/C ratios below $3.3 \times 10^{-3} \text{ mg} \cdot \text{mg}^{-1} \text{C}$ (Urabe and Watanabe, 1992; Sterner, 1993). However, ω3 PUFAs was explicitly linked to algal biosynthetic machinery rather than element allocation as P. Sufficient P in the medium can maintain high P/C ratio of algae but not ω3 PUFAs/C ratio. Grazers can be limited by ω3 PUFAs even when consuming high P/C food. Even though the P/C ratio ($5.0\text{--}10.0 \times 10^{-3} \text{ mg} \cdot \text{mg}^{-1} \text{C}$) of the cyanobacteria seems sufficient to the growth of *Daphnia*, low ω3 PUFAs/C ($0.011\text{--}0.043 \text{ mg} \cdot \text{mg}^{-1} \text{C}$) inhibit its growth. Moreover, P/C ratio was a less informatinal variance ($\Delta \text{AICc} = 9.343$ for P/C ratio versus 69.754 for ω3 PUFAs/C ratio) for explaining daphnid growth in the GLM. That is, the contribution of ω3 PUFAs/C ratio to the growth of *Daphnia* was greater than P/C ratio. Therefore, although both P and ω3 PUFAs are important nutrients for zooplankton growth, the indirect effects of elevated temperature and CO₂ on the growth of daphnids are mainly mediated by ω3 PUFAs/C ratio of phytoplankton.

Temperature and CO₂ significantly altered the quality of algal food fed on *Daphnia*. The effect of temperature on nutrient content of alga, considered from a cell quota view point, has been shown in the previous studies. An increase in the growth rate of algae generally accompanied with a decrease in their nutrient contents (Urabe et al., 2002; Dickman et al., 2008). The minimal cell quota of algae for P decreased with increasing growth temperature (Fig. 2). This decrease is consistent with a requirement for less P at higher temperatures (Rhee and Gotham, 1981). Temperature can also modify fatty acid saturation and alter PUFAs content of phytoplankton (Fig. 3). The thermo-adaptation mechanism via PUFAs alteration maintained the fluidity of cellular membrane and the metabolic process at high temperature (Adlerstein et al., 1997). Rising temperature resulted in the reduction in the relative content of the more unsaturated fatty acids of microalgae, particularly the trienoic fatty acids (Sushchik et al., 2003; Jiang and Gao, 2004). However, there was not a simple correlation of decreased unsaturation with higher temperature, because increasing temperature also caused

more subtle alterations in many algal species. For example, an increase in culture temperature from 10 to 25 °C led to an elevation in the relative proportions of linoleate and stearidonic acid (SDA) but a decrease in oleate in green alga *Selenastrum capricornutum* (McLarnon-Riches et al., 1998). In this study, elevated temperature increased ω 3 PUFAs/C of the green alga and the mixed culture while decreased that of the diatom and the cyanobacteria, indicating the species-specific effect of elevated temperature on PUFAs content (Fig. 3). The response in all algal cultures to elevated CO₂ is similar to reported responses in the previous studies. Under optimal nutrients supply conditions, elevated CO₂ increased autotroph biomass and decreased nutrients to C ratio due to a skewed accumulation of C relative to P (or nitrogen) (Urabe et al., 2003). Increase in CO₂ concentration from 0.03 to 2.0% resulted an increase in PUFA biosynthesis of the green alga *Chlamydomonas reinhardtii*. The fatty acid synthesis of the green algal species with higher unsaturation was repressed in low CO₂ condition, which allowed desaturation of preexisting fatty acids once CO₂ concentration increased (Pronina et al., 1998; Sato et al., 2003). Therefore, elevated temperature and CO₂ reduced P and/or ω 3 PUFAs/C ratios of algae, which generally became less favorable for daphnids growth.

It is necessary to point out the limitation of using a single clone of *Daphnia* as this study. The adaptation of *Daphnia* to local habitat conditions can lead to the differences in *Daphnia* sensitivity to environmental stressors (Tessier and Woodruff, 2002). This implied the possibility that using *Daphnia* isolated from different habitats or previously adapted to high temperature would produce different results. Although the observed adaptation was too faint to alter the response trend of daphnids species to temperature (Sikora et al., 2014), the current study cannot exclude this possibility. Moreover, the interspecific difference may limit the extrapolation of the findings from daphnids to other non-daphnids species. Compared with other cladoceran species, daphnids are large-bodied species (>2 mm) and generally more sensitive to temperature and algal food quality (Zaret, 1980; Abrusán, 2004). The response pattern of daphnids to temperature or CO₂ was similar to non-daphnids species, although the variation degree of daphnids' growth rate was larger than that of non-daphnids species (e.g. *Daphnia* vs. *Simocephalus*, *Daphnia* vs. *Bosmina*) (Masclaux et al., 2009; Shi et al., 2015). In addition, non-cladoceran species such as copepods and rotifers grow in a wider temperature range from 5 to 35 °C and require less phosphorus compared to daphnids (Pawlowski et al., 2018; Carrillo et al., 2001). In warmer freshwaters or seasons, large cladocerans *Daphnia* are typically replaced by smaller zooplankton especially copepods and rotifers, in which cyanobacterium with low P/C ratio are dominant in algal community (Zhang et al., 2018). This implies that non-cladoceran species are greater tolerant to high temperature and low quality food than daphnids species. The combined effect of elevated temperature and CO₂ on smaller non-daphnids species may be weaker than *Daphnia magna* studied in this work. Nevertheless, the adaptation of daphnids and the response of non-daphnids zooplankton to elevated temperature and CO₂ should be further investigated in the future to precisely predict the effect of climate change on freshwater planktonic communities.

5. Conclusions

The efficiency with which carbon and energy are transferred across the phytoplankton–zooplankton interface is highly variable, and these trophic levels can even become uncoupled in lakes (Brett and Muller-Navarra, 1997; Micheli, 1999). In eutrophic lakes, for example, the inhibition of carbon and energy transfer at the phytoplankton–zooplankton link usually results in an accumulation of phytoplankton biomass instead of sustaining production of higher trophic levels, such as fish (Müller-Navarra et al., 2000). Climate change may have powerful direct and indirect effects which may constrain the carbon transfer between phytoplankton and zooplankton.

As shown in this study, elevated temperature and CO₂ increased the biomass but increased stoichiometric imbalance and fatty acids of phytoplankton, which resulted in a decrease of zooplankton growth. The combined effects are mainly due to changes in food quality (fatty acid content) rather than the direct effects of CO₂ or temperature. However, the phytoplankton-mediated effects of elevated temperature and CO₂ and the direct effect of elevated temperature on zooplankton growth may entangle the interaction and carbon transfer between phytoplankton and zooplankton. Hence, more studies investigating the combined effects of elevated temperature and CO₂ are required to better predict responses of the phytoplankton–zooplankton interaction under different climate change scenarios. As temperature and CO₂ will continue to rise concurrently (IPCC, 2013), further studies investigating these abiotic factors in combination will provide important information on the ecological consequences of climate change in freshwater.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.12.180>.

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